SUMMARY STATEMENT
( Privileged Communication ) *Release Date:* 10/27/2003

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*Application Number:* 1 U01 NS048122-01

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*Review Group:* NSD-C
Neurological Sciences and Disorders C

*Meeting Date:* 10/16/2003
*Council:* JAN 2004
*Requested Start:* 04/01/2004

*RFA/PA:* PAR02-139
*PCC:* PORTELCN

*Project Title:* Therapeutic Interventions in Peripheral Neuropathy

*SRG Action:* Priority Score: 227 Percentile: 45.2
*Human Subjects:* 10-No human subjects involved
*Animal Subjects:* 30-Animals involved - no SRG comments or concerns noted

| Project Year | Direct Costs Requested | Estimated Total Cost |
|---|---|---|
| 1 | 545,788 | 715,027 |
| 2 | 522,108 | 684,004 |
| 3 | 573,975 | 751,954 |
| 4 | 701,414 | 918,909 |
| 5 | 859,446 | 1,125,944 |
| TOTAL | 3,202,731 | 4,195,838 |

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

**1 U01 NS048122-01** **GLASS, JONATHAN D**

**RESUME AND SUMMARY OF DISCUSSION:** This is a new application to establish a cooperative agreement under the NINDS program in Translational Research for the purposes of identifying calpain inhibitors that may be good candidate therapeutics for the treatment of chemotherapy-induced peripheral neuropathies. The reviewers regarded this application as highly responsive to the program announcement. Strengths of the application include its potentially very great significance -- there is a tremendous need for treatments for peripheral neuropathies of all types, the potential that compounds identified by the proposed approach would be useful for multiple types of neuropathy, the excellence of the experimental model, the access to a large family of compounds that could be tested, and the talents and expertise of the team of investigators. The major weakness of the application was that the lead compound appears to have too low an in vivo potency to be a very good candidate and the second generation compounds appeared to be even less potent. During the discussion, some panel members commented that the preliminary neuropathological data in figures 1 and 2 were not very compelling. It was also noted that, for chemotherapy-induced neuropathies, it may be possible to begin treatments before the neuropathy occurs, and therefore it was considered important to consult with oncologists for advice on how best to determine that the calpain inhibitors do not interfere with chemotherapeutic actions. The proposal was recommended with very good enthusiasm.

**DESCRIPTION (provided by applicant):** Peripheral neuropathies (PN) constitute a major category of neurologic disease causing progressive numbness, pain, and weakness in millions of people worldwide. The sequelae of peripheral neuropathy result in significant neurological morbidity and billions of dollars spent in direct medical costs and loss of productivity. Peripheral neuropathy is also the most frequent neurotoxic side effect of chemotherapeutic agents, resulting in dose reduction or cessation of otherwise effective cancer therapies. Chemotherapy induced peripheral neuropathy (CIPN), like many other neuropathies, is characterized by degeneration of axons. Axonal degeneration is associated with activation of calcium-activated cysteine proteases, calpains. Inhibition of calpains prevents axonal degeneration in experimental models. We developed a clinically relevant animal model of peripheral neuropathy caused by administration of Taxol, a commonly used chemotherapeutic drug for solid cancers. Mice treated with Taxol develop a sensory neuropathy typical of the human condition. Systemic administration of an experimental calpain inhibitor, AK295, prevents Taxol neuropathy in both short and long term studies. The broad, long-term objective of this proposal is to further characterize AK295 and develop enough pre-clinical data in order to seek approval as a treatment for human neuropathy. The Aims are to 1) develop an assay method to measure blood and tissue levels of AK295, 2) optimize the dose and schedule of AK295 for prevention of neuropathy, and test the oral bioavailability of AK295, 3) assure that AK295 will not interfere with the primary anti-cancer effects of Taxol, and 4) prepare an IND application for the FDA for testing AK295 in humans. An additional Aim will be to test our library of calpain inhibitors, comparing them to AK295 for efficacy, toxicity, and bioavailability. Successful completion of these Aims will provide a potential treatment for neuropathy, where no current treatment now exists.

**CRITIQUE 1:**

IMPORTANCE. The goal of this project is to conduct a series of preclinical studies on the experimental calpain inhibitor, AK295, as a means of evaluating its potential as a drug for preventing chemotherapy-induced peripheral neuropathy (PN). PN is a common side effect observed in the treatment of breast cancer patients with Taxol. Therefore, the identification of a drug that can effectively reduce PN while not interfering with the primary anti-cancer effects of Taxol would represent an important improvement in the treatment of breast cancer. Furthermore, since PN is observed in a variety of other diseases, including diabetes mellitus, HIV infection, and other types of cancer chemotherapy, the results of this study may lead to a treatment strategy that extends beyond the treatment of breast cancer.

APPROACH. The research described in this grant application is an excellent example of the intent of the program announcement, which is focused on funding projects aimed at therapy development

necessary to begin clinical testing of a drug that is of importance to the clinical mission of the NINDS. In this application, the PI has provided interesting preliminary data regarding the ability of the peptide analog, AK295, to reduce Taxol-induced peripheral neuropathy. In addition, preliminary data was provided to demonstrate that the team of investigators has an interesting pipeline of compounds that can also be evaluated for their efficacy in reducing induced-induced PN. There are four specific aims described in this project. The first specific aim focuses on the development of a series of analytical methods for evaluating the in vivo properties of AK295. This includes the development of HPLC methods for determining the enantiomeric purity and stability (i.e., epimerization of the Aby residue in AK295) in vivo, as well as the development of ELISA and LC/MS methods for metabolite analysis. The second specific aim involves evaluating the pharmacokinetics of AK295 using different routes of administration. This includes comparing subcutaneous and oral routes of administration. The third specific aim is essentially a continuation of specific aim 2, but involves looking at the ability of AK295 to prevent induced-induced PN using the different routes of administration. Specific aim 4 involves a series of in vitro screens aimed at identifying new calpain inhibitors that work as well or better than AK295. The analogs that will be evaluated will come from a compound library reported by Dr. Powers, the Co-PI of this project, in the Journal of Medicinal Chemistry in 1996.

Although the proposed research contains several strong points, there are a number of concerns regarding the in vivo properties of AK295, and its structural congeners, that diminish the level of enthusiasm for this proposal. The first (and major) concern is related to the ability of AK295 to cross the cell membrane and inhibit calpain in vivo. For example, the Ki of AK295 for inhibiting calpain II in vitro is 41 nM. However, the IC50 for inhibiting calpain II in cell culture (i.e., the platelet membrane permeability assay) is 45 uM, which represents a 1000-fold lower potency in the cell culture assay (i.e., the IC50 platelet permeability assay/Ki in vitro ratio = 1097). It is not clear why this is the case since the lipophilicity of AK295 is within a range that is expected to readily cross cell membranes (calculated log P = 2.03 at pH = 7.4 using the program ClogD). The low cellular uptake of AK295 is also consistent with the high doses required to demonstrate activity in the mouse model of Taxol-induced PN (48 mg/kg s.c., or 24 mg/kg/day using an Alzet minipump delivery system). Since this compound is likely to excreted rapidly through the hepatobilliary and renal systems, the oral dose of AK295 is predicted to be much higher and will likely lead to tissue toxicity in these organs. In addition, the second generation compounds (18, 19, and 35) are likely to have a lower in vivo potency since the IC50 platelet permeability assay/Ki in vitro ratios are 1364 (compound 18), 4600 (compound 19) and 6470 (compound 35).

A second (albeit minor) concern is related to the use of AK295 to prevent Taxol-induced PN. Taxol is currently being replaced with taxotere in the treatment of breast cancer since it has a much lower tendency to cause PN. The greatest clinical impact of a drug to prevent PN is in the area of diabetes-induced PN, which represents a major health problem in the US. The PI should have made a stronger case in stating that the induced-induced NP assay is an appropriate animal model for studying PN in general, and that the outcome of this project will have its greatest clinical effect in the treatment of diabetes.

MILESTONES. The PI has identified a clear list of tasks and milestones on page 35 of the grant application. However, the PI has not clearly outlined the criteria for success needed for justifying a clinical trial of AK295 or one of its structural congeners.

INVESTIGATORS. The PI and Co-PI lead a strong team of investigators and are very capable of conducting the research described in this project.

ENVIRONMENT. Adequate for the proposed research project.

PROTECTIONS. No concerns.

DATA AND RESOURCE SHARING. No data and resource sharing plan was provided.

BUDGET. The percent effort of the PI is somewhat high (50%) for someone in a supervisory role. It should be reduced to 25%.

**CRITIQUE 2:**

This is an interesting application that proposes to further characterize AK295 and develop enough pre-clinical data to seek approval for using AK295 to block chemotherapy induced peripheral neuropathy. The major strengths of the application are the data Dr. Glass has already generated showing that AK295 can attenuate Taxol induced peripheral neuropathy as assessed by both histological and a behavioral measure (rotarod performance). There are several concerns however about the in vitro vs. in vivo potency of AK295 and the adequacy and completeness of the animal model employed to assess the in vivo ability to AK295 and other calpain inhibitors not to interfere with Taxol's anti-tumor effects while preventing the chemotherapy induced peripheral neuropathy.

First, the major issue of any drug that will be use concomitantly with an anti-tumor agent such as Taxol will be that the drug in no way interfere with the tumoricidal actions of the chemo-therapeutic agent. In the present proposal the sole assay the PI proposes to use to assess that AK295 does not block any of the tumoricidal actions of Taxol is an in vitro assay of Taxol sensitive human breast cancer cells (it is not stated what specific cell line of human breast cancer cells will be used). The sole use of an in vitro tumor assay along with the demonstration that AK295 has higher potency in vitro than in vivo raises a concern that the low concentrations of AK295 used in vitro will not accurately predict the effects of the high concentrations that will be needed to be used in vivo. A stronger approach to addressing the issue of whether calpain inhibitors do not in any way interfere with the anti-tumorigenic actions of Taxol while still having the anti-CIPN actions would be to use an in vivo model where breast cancer cells are injected into the mice and allowed to grow and then test the ability of AK295 to block Taxol induced peripheral neuropathy while not interfering with Taxol's ability to kill the tumor. The major point here is that in vivo and in vitro data has demonstrated that many tumoricidal agents and regimes may depend on their on actions on endogenous host stromal cells (fibroblasts, endothelial cells, etc) as much if not more than their direct actions on the tumor cells themselves. Thus, it is critical that the PI has in place an in vivo model (and preferably several in vivo model where several tumor cell lines can be evaluated) where the anti-tumoricidal actions of AK295 actions on stromal and tumor cells can be simultaneously monitored.

Second, given the importance of the above issue it would strengthen the application if an oncologist was added as a consultant. Thus, while it may be clear to a neurologist that CIPN can be a serious and highly debilitating condition, for an oncologist the tumor is frequently the main if not the only target considered. Thus, if AK295 or another calpain inhibitor does prevent CIPN, collaborating with a clinician with this perspective may allow earlier implementation and acceptance of this therapy in an oncological setting.

A third major concern is that the behavioral assessment of chemotherapy induced peripheral neuropathy relies solely on rotarod performance even though motor performance, numbness and pain are frequent symptoms of chemotherapy induced neuropathy. The application would be much stronger and more predictive of the ability of AK295 to attenuate the various aspects of chemotherapy induced neuropathy if other behavioral measures of pain (mechanical, thermal and chemical) and general activity (running wheel or grid crossings) were included in the behavioral assessment.

Lastly, it would be helpful if the PI could more thoroughly review the current state of calpain inhibitors being used in any human clinical trials. The major review cited by the author (Wang et al, 1994) entitled "Calpain inhibitors: an overview of its therapeutic potential" is now nearly a decade old and a obvious question is what neuroprotective trials for any neurological condition have been conducted to date and if none have been conducted what are the issues which prevent such trials from taking place? Providing a more thorough and comprehensive discussion of the potential advantages, drawbacks and

side effects of calpain inhibitors, whether it be in chemotherapy induced neuropathy or other neurological disorders, would significantly strengthen the application.

SIGNIFICANCE: The proposed studies address the important question of how chemotherapy induced peripheral neuropathy (CIPN) can be reduced. CIPN occurs frequently and can limit the dose of chemotherapy the patient can receive so CIPN can impact both quality of life and survival of the patient.

APPROACH: The overall approach taken by the PI is sound although what appears to be missing is balancing in vitro vs. in vivo effects and the perspective of an oncologist where the tumor is the main if not the only target. As CIPN is one of the only neuropathies where a neuroprotective therapy could be initiated before the neuropathy develops, for this therapy to be translated into clinical reality there must be acceptance by the oncologist. For this reason, it would be helpful if the proposal would also include an in vivo tumor assay and involve an oncologist who can assess the quality and end-points that will be used in measuring potential CIPN therapies effects on tumor killing and metastasis.

INNOVATION: The techniques outlined by the PI are not particularly innovative as calpain inhibitors have been around for well more than a decade. What is innovative about this proposal is that the PI is attempting to develop a therapy for treating a condition that currently has only non-mechanism based therapies in place to deal with the CIPN long after it has been induced.

INVESTIGATOR: Dr. Glass is a neurologist at Emory and received excellent training in the clinical understanding and assessment of peripheral neuropathies at Johns Hopkins University

ENVIRONMENT: While the general research environment at Emory University is good it would be useful if the PI could include interactions with an oncologist and members of a Cancer Center whose primary target is the tumor.

BUDGET: The budget appears to be high given the relatively limited number of experiments that are proposed.

ANIMAL WELFARE: The use of animals is clearly justified as it is the only approach currently available to experimentally determine the mechanisms that give rise to CIPN.

**THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:**

**VERTEBRATE ANIMALS (Resume): ACCEPTABLE.**

**COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.**

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## MEETING ROSTER

### Neurological Sciences and Disorders C
### National Institute of Neurological Disorders and Stroke Initial Review Group
### NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE
### NSD-C
### October 16, 2003 - October 17, 2003

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.